



Certificate of Analysis

Standard Reference Material[®] 931f

Liquid Absorbance Standard for Ultraviolet and Visible Spectrophotometry

This Standard Reference Material (SRM) is intended primarily for critical evaluation of daily working standards used in spectrophotometry and for use as an accuracy check of the photometric scale of spectrophotometers that provide a narrow effective spectral bandpass not to exceed the following: 2.2 nm at 302 nm, 2.9 nm at 395 nm, 4.8 nm at 512 nm, and 12.3 nm at 678 nm [1].

SRM 931f consists of three kits, each comprising three cobalt-nickel solutions (liquid filters) and a blank solution (12 ampoules total). The three liquid filters, referred to as levels I, II, and III, have nominal absorbances of 0.3, 0.6, and 0.9 at three certification wavelengths. The SRM is certified for net absorbance at four specific spectral wavelengths for a 10.00 mm measurement pathlength (see SRM Filter Preparation). Approximately 10 mL of liquid is individually flame-sealed into each glass ampoule, which has been prescored for easy opening. Each kit of four ampoules is packaged in a tray, with three trays in each box.

The certified net absorbances, corrected for the absorbance due to the blank solution, are given in Table 1 for the three liquid filters at four wavelengths for a 10.00 mm pathlength cell and a temperature of 21 °C ± 1 °C. The uncertainties of the certified values include all known sources of random and possible systematic errors (see Certification of Net Absorbance).

Table 1. Certified Absorbances and Uncertainties at Nominal Wavelengths

Liquid Filter	Nominal Wavelength, (nm)			
	302	395	512	678
Level I	0.2928 ± 0.0017	0.3109 ± 0.0017	0.2996 ± 0.0017	0.1170 ± 0.0012
Level II	0.5956 ± 0.0028	0.6257 ± 0.0029	0.5958 ± 0.0029	0.2359 ± 0.0014
Level III	0.9257 ± 0.0042	0.9173 ± 0.0041	0.8838 ± 0.0041	0.3465 ± 0.0018

Note: All certified values have been corrected for the absorbance due to the blank solution, and normalized to a pathlength of 10.00 mm.

The overall coordination of technical measurements leading to certification was performed by G.W. Kramer of the NIST Analytical Chemistry Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by R.J. Gettings.

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Certificate Issue Date: 4 February 1999

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Filter solution preparation and original transmittance measurements leading to certification were performed by M.V.

Statistical analysis was performed by H-k Liu of the NIST Statistical Engineering Division.

Certified Value Uncertainties: The uncertainties listed in Table I are expanded uncertainties, calculated according to the CIPM approach [2] at the 95 % level of confidence. These uncertainties include contributions due to: random variation in the spectrophotometer measurements; an ambient temperature variation of ± 1 °C; finite bandwidth effects; and baseline bias.

Expiration of Certification: The certified absorbances are valid until **31 December 2007** within the uncertainties stated, provided the SRM is handled and stored in accordance with the Instructions for Use and Caution to User sections of this certificate. Verification is performed periodically at NIST on control samples from this and previous issues of SRM 931 to confirm stability.

SRM Filter Preparation: The filters were prepared by dissolving high purity cobalt and nickel in a mixture of reagent grade nitric and perchloric acids and diluting in distilled water. The blank was prepared as 9 mL/L perchloric acid in distilled water. The absorbance spectrum of the resulting solutions is shown in Figure 1. The maxima at 302 nm and 512 nm are due to absorbance by NO_3^- and $\text{Co}(\text{H}_2\text{O})_6^{+2}$ respectively. The maximum at 395 nm and the plateau in the 650 nm to 700 nm range are due to $\text{Ni}(\text{H}_2\text{O})_6^{+2}$. The pH of these solutions is approximately 1.

Certification of Net Absorbance: The transmittance measurements leading to the certification of this SRM were performed at an ambient temperature of $21\text{ °C} \pm 1\text{ °C}$ using the NIST high accuracy spectrophotometer. The design and construction of this instrument have been described previously [3]. The instrument is a primary transmittance standard; its accuracy has been verified using the double aperture radiation addition principle. The effective spectral bandpass used to determine the certified values was 1.6 nm. The transmittance measurements were made by producing the vertical image of the slit (about 8 mm X 2.0 mm), using convergent beam geometry with an aperture ratio f/10, in the middle of the entrance face of the sample cuvette. The cuvette was oriented in a position perpendicular to the incident light beam.

The liquid filter solutions were calibrated at the wavelengths and conditions indicated by measuring the transmittance, T , of the blank and level I, II, and III solutions referenced to air. Each transmittance measurement is calculated from a measurement of the intensity transmitted through a 10 mm cuvette containing the SRM solution and bracketing measurements of the intensity transmitted through an empty cuvette holder, with a settling time of approximately 5 s and a signal integrating time of approximately 1 s for each measurement.

For the certification measurements using cuvettes with a pathlength of $10.000\text{ mm} \pm 0.0005\text{ mm}$, 18 ampoules were randomly selected from each of the four solutions (blank and three absorbance levels). Randomized measurements were made in four runs on each of three days. In each run, six unknown samples (SRM 931f) and one control sample (SRM 931e) were measured in triplicate. Two samples from three of the four levels were measured in each run. A blank was run in each cuvette on each day.

The values of T were used to calculate the corresponding values of the apparent absorbance, A , using the relationship $A = -\log_{10}T$. The net absorbances were obtained by subtracting the apparent absorbance of the blank solution from the apparent absorbances calculated for solutions I, II, and III and corrected to a 10.000 mm pathlength and a 21.0 °C temperature. The certified net absorbances are the grand mean values for the three levels and four wavelengths.

Temperature Dependence: Absorbances at various temperatures (17 °C to 35 °C) may be corrected to the certification temperature using the equation:

$$A_{21} = A_t/[1+C_A(t-21)]$$

where: A_{21} = Absorbance corrected to 21.0 °C

A_t = Measured absorbance at temperature t (°C)

C_A = Fractional change in absorbance per °C

t = Sample chamber temperature

The values of C_A at the four certification wavelengths are given in Table 2.

Note: At wavelength 302 nm, absorbance decreases with increasing temperature; at the other wavelengths, absorbance increases with increasing temperature.

Table 2. Temperature Coefficients at Certification Wavelengths

Nominal Wavelength (nm)	C _A
302	- 0.0014
395	+ 0.0014
512	+ 0.0018
678	+ 0.0014

Wavelength Accuracy and Bandpass Requirements: Due to the spectral features in the absorbance spectrum (see Figure 1), the absorbances of these liquid filters will depend not only on the accuracy of the photometric scale, but also on the wavelength accuracy and the spectral bandpass of the spectrophotometer. NIST SRM 2034, Holmium Oxide Solution Wavelength Standard, is recommended for calibrating the wavelength scale. In addition, for those spectrophotometers having a hydrogen (H₂) or deuterium (D₂) source, the two emission lines at 486.1 nm and 656.3 nm (H₂) or 486.0 nm and 656.1 nm (D₂) may provide a convenient check at these wavelengths. To ensure that the measured absorbances are not significantly different from the certified values, the wavelength scale of the spectrophotometer should be calibrated to within ± 0.5 nm, and the effective spectral bandpass should not exceed 2.2 nm, 2.9 nm, 4.8 nm, and 12.3 nm at 302 nm, 395 nm, 512 nm, and 678 nm, respectively.

Caution to User: Temperature affects the absorbance of these standards. If the sample chamber differs by more than $\pm 1^\circ\text{C}$ from room temperature during the calibration, either the data should be collected quickly or the sample be allowed to equilibrate to the known sample chamber temperature.

Instructions for Use: SRM 931f should be kept in the original sealed ampoules until use. Once opened, the solutions should be used immediately. No attempt should be made to reseal the ampoules.

The instructions below are for use with the standard 10 mm pathlength rectangular cuvette and apply to either single beam or double beam spectrophotometers. For validation of spectrophotometric systems used in various automated instruments, the user should refer to the instruction manual for the particular instrument.

- Step 1. Reserve at least one 10 mm cuvette of known pathlength and uncertainty for all SRM measurements. Cuvettes should be clean, free of scratches, and fitted with ground-glass or Teflon^{®1} stoppers. A single cuvette (into which a liquid filter can be pipetted) may be used and left in place in the sample chamber of the spectrophotometer to minimize sources of variability due to cuvette pathlength, positioning, etc. Alternatively, the use of four stoppered cuvettes permits randomized replication over the course of a day.
- Step 2. Mark each cuvette to assure reproducible orientation in the spectrophotometer in case the cuvette is removed and replaced during the calibration procedure.
- Step 3. Rinse each cuvette repeatedly with deionized organic-free water, making apparent absorbance readings (referenced to air) at 302 nm after each rinse, until constant absorbance readings are obtained. Borosilicate Pasteur-type pipettes fitted with rubber bulbs are recommended for transferring solutions to and from the cuvettes. (Commercially available soft glass pipettes may contain residual amounts of ultraviolet absorbing material, but may be used after proper cleaning. Several rinses, first with isopropyl alcohol and then with deionized organic-free water, are generally adequate.)
- Step 4. Using the liquid filter solutions provided, measure the apparent absorbance of the blank and of solutions I, II, and III against air in the following manner. Assure that the solutions and cuvettes are temperature-equilibrated to the laboratory before measurement. Record the appropriate temperature. Shake each ampoule before opening to remix any condensate that may have been collected in the neck. (The ampoules have been prescored directly below the colored band to facilitate opening.) Rinse the sample cuvette with at least two complete fillings of the SRM solution from each 10 mL ampoule before taking final apparent

¹Certain commercial materials and equipment are identified to adequately specify the experimental procedure. Such identification does not imply a recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for this purpose.

absorbance readings for that ampoule at nominal wavelengths of 302 nm, 395 nm, 512 nm, and 678 nm, using an effective spectral bandpass not to exceed 2.2 nm, 2.9 nm, 4.8 nm, and 12.3 nm, respectively. (Unless wavelength accuracy to within 0.5 nm can be verified with SRM 2034, absorbance maxima in the vicinity of the shortest three wavelengths should be used to test the absorbance accuracy of the instrument independently of the wavelength accuracy.) Solutions from the rinses should be collected for proper disposal. If four cuvettes are used, replication is recommended with removal and replacement of cuvettes between runs and a randomized run order for the four solutions. **Validation must be performed the same day when using solutions prepared in stoppered cuvettes due to the possibility of evaporative losses.**

- Step 5. For each wavelength and replication, correct all results to a pathlength of 10.00 mm, and subtract the apparent absorbance of the blank from the apparent absorbances obtained for solutions I, II, and III to yield the net absorbances for the three levels. Use the temperature dependence equation and coefficients given above to correct the measured net absorbance values from the temperature recorded in Step 4 to the certification temperature of 21 °C. The temperature-corrected net absorbances should agree with the certified values within a tolerance determined by overlap of the certified uncertainties and the uncertainty of the user's experimental method. The latter uncertainty may typically include replication uncertainty (if determined), the cuvette pathlength uncertainty, and the specified accuracy uncertainty for the spectrophotometer. Consult the manufacturer of the instrument if adequate agreement is not achieved.
- Step 6. Upon completion of measurements, empty the cuvettes and any material remaining from the four ampoules into a container for proper disposal.

REFERENCES

- [1] Burke, R.W., Deardorff, E.R., and Menis, O., J. Research Nat. Bur. Stand. **76A**, pp. 469-482, (1972).
- [2] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland, (1993): see also Taylor, B.N. and Kuyatt, C.E., "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington DC, (1994).
- [3] Mavrodineanu, R., J. Research Nat. Bur. Stand. **76A**, pp. 405-425, (1972): see also NBS Special Publication 378, *Accuracy in Spectrophotometry and Luminescence Measurements*, Mavrodineanu, R., Shultz, J.I., and Menis, O., Editors, U.S. Government Printing Office, Washington DC, (1973).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: Telephone (301) 975-6776 (select "Certificates"), Fax (301) 926-4751, e-mail srminfo@nist.gov, or via the Internet <http://ts.nist.gov/srm>.

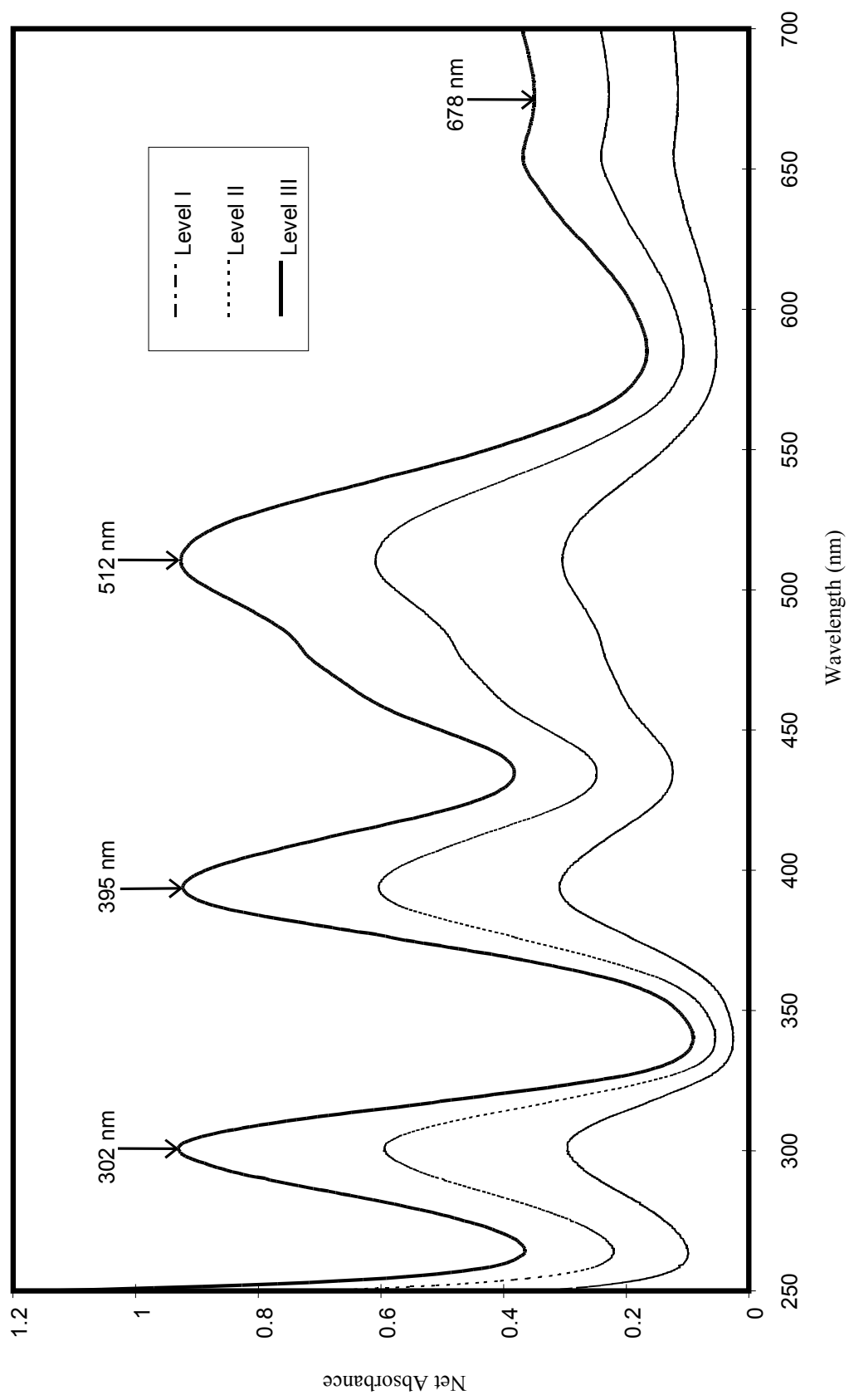


Figure 1. Absorbance Spectrum of SRM 931f